

Claims

- 1 1. Substantially pure DNA encoding an *Rps*
polypeptide.
- 1 2. The DNA of claim 1, wherein said DNA contains
2 the *RPS2* gene.
- 1 3. The DNA of claim 1, wherein said DNA is genomic
2 DNA.
- 1 4. The DNA of claim 1, wherein said DNA is cDNA.
- 1 5. The DNA of claim 1, wherein said DNA is of a
2 plant of the genus *Arabidopsis*.
- 1 6. Substantially pure DNA having the sequence of
2 Fig. 2, or degenerate variants thereof, and encoding the
3 amino acid sequence of open reading frame "a" of Fig. 2.
- 1 7. Substantially pure DNA having about 50% or
2 greater sequence identity to the DNA sequence of Fig. 2.
- 1 8. The DNA of claim 1 or 2, wherein said DNA is
2 operably linked to regulatory sequences for expression of
3 said polypeptide; and
4 wherein said regulatory sequences comprise a
5 promoter.
- 1 9. The DNA of claim 8, wherein said promoter is a
2 constitutive promoter.

1 10. The DNA of claim 8, wherein said promoter is
2 inducible by one or more external agents.

1 11. The DNA of claim 8, wherein said promoter is
2 cell-type specific.

1 12. A cell which contains the DNA of claim 1.

1 13. The cell of claim 12, said cell being a plant
2 cell.

1 14. The plant cell of claim 13, said plant cell
2 being resistant to disease caused by a plant pathogen
3 carrying an avirulence gene generating a signal recognized
4 by an Rps polypeptide.

1 15. The plant cell of claim 14, said plant pathogen
2 carrying an *avrRpt2* gene.

1 16. The plant cell of claim 14, said plant cell
2 being from the group of plants comprising *Arabidopsis*,
3 tomato, soybean, bean, maize, wheat, and rice.

1 17. The plant cell of claim 14, said plant pathogen
2 being *Pseudomonas syringae*.

1 18. The plant cell of claim 13, wherein said plant
2 cell further contains an *avrRpt2* gene operably linked to
3 regulatory sequences; and
4 wherein said regulatory sequences comprise a
5 promoter.

1 19. The plant cell of claim 18, wherein said
2 promoter is a constitutive promoter.

1 20. The plant cell of claim 18, wherein said
2 promoter is inducible by one or more external agents.

1 21. The plant cell of claim 18, wherein said
2 promoter is cell-type specific.

1 22. A transgenic plant which contains the DNA of
2 claim 1 integrated into the genome of said plant, wherein
3 said DNA is expressed in said transgenic plant.

1 23. A transgenic plant which contains the DNA of
2 claim 8 integrated into the genome of said plant, wherein
3 said DNA is expressed in said transgenic plant.

1 24. A transgenic plant generated from the plant
2 cell of claim 18 wherein said DNA and said avrRpt2 gene are
3 expressed in said transgenic plant.

1 25. A seed from a transgenic plant of claim 22.

1 26. A seed from a transgenic plant of claim 23.

1 27. A seed from a transgenic plant of claim 24.

1 28. A cell from a transgenic plant of claim 22.

1 29. A cell from a transgenic plant of claim 23.

1 30. A method of providing resistance to a plant
2 pathogen in a plant, said method comprising:

3 producing a transgenic plant cell comprising the DNA
4 of claim 1 integrated into the genome of said transgenic
5 plant cell and positioned for expression in said plant cell;
6 and
7 growing a transgenic plant from said plant cell
8 wherein said DNA is expressed in said transgenic plant.

1 31. A method of detecting a resistance gene in a
2 plant cell, said method comprising:
3 contacting the DNA of claim 1 or a portion thereof
4 greater than about 18 nucleic acids in length with a
5 preparation of genomic DNA from said plant cell under
6 hybridization conditions providing detection of DNA
7 sequences having about 50% or greater sequence identity to
8 the sequence of Fig.2.

1 32. A method of producing an Rps2 polypeptide
2 comprising:
3 providing a cell transformed with DNA encoding an
4 Rps2 polypeptide positioned for expression in said cell;
5 culturing said transformed cell under conditions for
6 expressing said DNA; and
7 isolating said Rps2 polypeptide.

1 33. A method of providing, in a transgenic plant,
2 resistance to a plant pathogen, said method comprising:
3 producing a transgenic plant cell comprising the DNA
4 of claim 8 integrated into the genome of said transgenic
5 plant cell and positioned for expression in said plant cell;
6 and
7 growing said transgenic plant from said plant cell
8 wherein said DNA is expressed in said transgenic plant.

1 34. A method of providing, in a transgenic plant,
2 resistance to a plant pathogen, said method comprising:
3 growing said transgenic plant from the plant cell of
4 claim 18 wherein said DNA and said *avrRpt2* gene are
5 expressed in said transgenic plant.

1 35. A method of isolating a disease resistance gene
2 or portion thereof in plants having sequence identity to
3 *RPS2*, said method comprising:
4 amplifying by PCR said disease resistance gene or
5 portion thereof using oligonucleotide primers wherein said
6 primers
7 (a) are each greater than 13 nucleotides in
8 length;
9 (b) each have regions of complementarity to
10 opposite DNA strands in a region of the nucleotide sequence
11 of Fig. 2; and
12 (c) optionally contain sequences capable of
13 ~~producing restriction enzyme cut sites in the amplified~~
14 ~~product; and~~
15 isolating said disease resistance gene or portion
16 thereof.

1 36. A substantially pure *Rps2* polypeptide.

1 37. The polypeptide of claim 32, comprising an
2 amino acid sequence substantially identical to an amino acid
3 sequence shown in Fig. 2.

1 38. A vector comprising the DNA of claim 1, said
2 vector being capable of directing expression of the peptide
3 encoded by said DNA in a vector-containing cell.

1 39. A vector comprising the DNA of the *avrRpt2* gene
2 operably linked to regulatory sequences wherein said
3 regulatory sequences comprise a promoter.

1 40. A vector comprising the DNA of claim 1 and the
2 DNA of the *avrRpt2* gene operably linked to regulatory
3 sequences wherein said regulatory sequences comprise a
4 promoter.

1 41. A substantially pure oligonucleotide comprising
2 the sequence:

3 5' GGNATGGGNGGNNTNGGNAARACNAC 3', wherein N is A, T,
4 G, or C; and R is A or G.

1 42. A substantially pure oligonucleotide comprising
2 the sequence:

3 5' NARNGGNARNCC 3', wherein N is A, T, G or C; and R
4 is A or G.

1 43. A substantially pure oligonucleotide comprising
2 the sequence:

3 5' NCGNGWNGTNAKDAWNCGNGA 3', wherein N is A, T, G or
4 C; W is A or T; D is A, G, or T; and K is G or T.

1 44. A substantially pure oligonucleotide comprising
2 the sequence:

3 5' GGWNTBGGWAARACHAC 3', wherein N is A, T, G or C;
4 R is G or A; B is C, G, or T; H is A, C, or T; and W is A or
5 T.

1 45. A substantially pure oligonucleotide comprising
2 the sequence:

3 5' TYGAYGAYRTBKRBRA 3', wherein R is G or A; B is C,
4 G, or T; D is A, G, or T; Y is T or C; and K is G or T.

1 46. A substantially pure oligonucleotide comprising
2 the sequence:

3 5' TYCCAVAYRTCRTCNA 3', wherein N is A, T, G or C; R
4 is G or A; V is G or C or A; and Y is T or C.

1 47. A substantially pure oligonucleotide comprising
2 the sequence:

3 5' GGWYTBCCWYTBGCHYT 3', wherein B is C, G, or T; H
4 is A, C, or T; W is A or T; and Y is T or C.

1 48. A substantially pure oligonucleotide comprising
2 the sequence:

3 5' ARDGCVARWGGVARNCC 3', wherein N is A, T, G or C;
4 R is G or A; W is A or T; D is A, G, or T; and V is G, C, or
5 A.

1 49. A substantially pure oligonucleotide comprising
2 the sequence:

3 5' ARRTTRTCRTADSWRAWYTT 3', wherein R is G or A; W
4 is A or T; D is A, G, or T; S is G or C; and Y is C or T.

1 50. A recombinant plant gene comprising the DNA
2 sequence:

3 5' GGNATGGGNGGNNTNGGNAARACNAC 3', wherein N is A, T,
4 G or C; and R is A or G.

1 51. The gene of claim 50, further comprising the
2 sequence:

3 5' NARNGGNARNCC 3', wherein N is A, T, G or C; and R
4 is A or G.

1 52. The gene of claim 51, further comprising the
2 sequence:

3 5' NCGNGWNGTNAKDAWNCGNGA 3', wherein N is A, T, G
4 or C; W is A or T; D is A, G or T; and K is G or T.

1 53. A recombinant plant gene comprising a
2 combination of any two or more sequences of claims 50, 51,
3 and 52.

1 54. A substantially pure plant polypeptide
2 comprising the amino acid sequence:

3 Gly Xaa₁ Xaa₂ Gly Xaa₃ Gly Lys Thr Thr Xaa₄ Xaa₅,
4 wherein Xaa₁ is Met or Pro; Xaa₂ is Gly or Pro; Xaa₃ is Ile,
5 Leu, or Val; Xaa₄ is Ile, Leu, or Thr; and Xaa₅ is Ala or
6 Met.

1 55. A substantially pure plant polypeptide
2 comprising the amino acid sequence:

3 Xaa₁ Xaa₂ Xaa₃ Leu Xaa₄ Xaa₅ Xaa₆ Asp Asp Xaa₇ Xaa₈,
4 wherein Xaa₁ is Phe or Lys; Xaa₂ is Arg or Lys; Xaa₃ is Ile,
5 Val, or Phe; Xaa₄ is Ile, Leu, or Val; Xaa₅ is Ile or Leu;
6 Xaa₆ is Ile or Val; Xaa₇ is Ile, Leu, or Val; and Xaa₈ is
7 Asp or Trp.

1 56. A substantially pure plant polypeptide
2 comprising the amino acid sequence:

3 Xaa₁ Xaa₂ Xaa₃ Xaa₄ Xaa₅ Thr Xaa₆ Arg,
4 wherein Xaa₁ is Ser or Cys; Xaa₂ is Arg or Lys; Xaa₃ is Phe,
5 Ile, or Val; Xaa₄ is Ile, or Met; Xaa₅ is Ile, Leu, or Phe;
6 Xaa₆ is Ser, Cys, or Thr.

1 57. A substantially pure plant polypeptide
2 comprising the amino acid sequence:
3 Gly Leu Pro Leu Xaa₁ Xaa₂ Xaa₃ Xaa₄,
4 wherein Xaa₁ is Thr, Ala, or Ser; Xaa₂ is Leu or Val; Xaa₃
5 is Ile, Val, or Lys; and Xaa₄ is Val or Thr.

1 58. A substantially pure plant polypeptide
2 comprising the amino acid sequence:
3 Xaa₁ Xaa₂ Ser Tyr Xaa₃ Xaa₄ Leu,
4 wherein Xaa₁ is Lys or Gly; Xaa₂ is Ile or Phe; Xaa₃ is Asp
5 or Lys; and Xaa₄ is Ala, Gly, or Asn.

1 59. A method of isolating a disease-resistance gene
2 or fragment thereof from a plant cell, comprising:
3 (a) providing a sample of plant cell DNA;
4 (b) providing a pair of oligonucleotides having
5 sequence homology to a conserved region of an RPS disease-
6 resistance gene;
7 (c) combining said pair of oligonucleotides
8 with said plant cell DNA sample under conditions suitable
9 for polymerase chain reaction-mediated DNA amplification;
10 and
11 (d) isolating said amplified disease-resistance
12 gene or fragment thereof.

1 60. The method of claim 59, wherein said
2 amplification is carried out using a reverse-transcription
3 polymerase chain reaction.

1 61. The method of claim 59, wherein said reverse-
2 transcription polymerase chain reaction is RACE.

1 62. A method of identifying a plant disease-
2 resistance gene in a plant cell, comprising:
3 (a) providing a preparation of plant cell DNA;
4 (b) providing a detectably-labelled DNA sequence
5 having homology to a conserved region of an RPS gene;
6 (c) contacting said preparation of plant cell DNA
7 with said detectably-labelled DNA sequence under
8 hybridization conditions providing detection of genes having
9 50% or greater sequence identity; and
10 (d) identifying a disease-resistance gene by its
11 association with said detectable label.

1 63. The method of claim 62, wherein said DNA
2 sequence is produced according to the method of claim 59.

1 64. The method of claim 62, wherein said
2 preparation of plant cell DNA is isolated from a plant
3 genome.

1 65. A method of isolating a disease-resistance gene
2 from a recombinant plant cell library, comprising:
3 (a) providing a recombinant plant cell library;
4 (b) contacting said recombinant plant cell library
5 with a detectably-labelled gene fragment produced according
6 to the method of claim 59 under hybridization conditions
7 providing detection of genes having 50% or greater sequence
8 identity; and
9 (c) isolating a member of a disease-resistance gene
10 by its association with said detectable label.

1 66. A method of isolating a disease-resistance gene
2 from a recombinant plant cell library, comprising:

- 3 (a) providing a recombinant plant cell library;
4 (b) contacting said recombinant plant cell library
5 with a detectably-labelled oligonucleotide of any of claims
6 41-49 under hybridization conditions providing detection of
7 genes having 50% or greater sequence identity; and
8 (c) isolating a disease-resistance gene by its
9 association with said detectable label.

1 67. A recombinant plant polypeptide capable of
2 conferring disease-resistance wherein said plant polypeptide
3 comprises a P-loop domain or nucleotide binding site domain.

1 68. The recombinant plant polypeptide of claim 67,
2 wherein said polypeptide further comprises a leucine-rich
3 repeating domain.

1 69. A recombinant plant polypeptide capable of
2 conferring disease-resistance wherein said plant polypeptide
3 contains a leucine-rich repeating domain.

1 70. A plant disease-resistance gene isolated
2 according to the method comprising:

- 3 (a) providing a sample of plant cell DNA;
4 (b) providing a pair of oligonucleotides having
5 sequence homology to a conserved region of an RPS disease-
6 resistance gene;
7 (c) combining said pair of oligonucleotides with
8 said plant cell DNA sample under conditions suitable for
9 polymerase chain reaction-mediated DNA amplification; and
10 (d) isolating said amplified disease-resistance gene
11 or fragment thereof.

1 71. A plant disease-resistance gene isolated
2 according to the method comprising:
3 (a) providing a preparation of plant cell DNA;
4 (b) providing a detectably-labelled DNA sequence
5 having homology to a conserved region of an RPS gene;
6 (c) contacting said preparation of plant cell DNA
7 with said detectably-labelled DNA sequence under
8 hybridization conditions providing detection of genes having
9 50% or greater sequence identity; and
10 (d) identifying a disease-resistance gene by its
11 association with said detectable label.

1 72. A plant disease-resistance gene according to
2 the method comprising:
3 (a) providing a recombinant plant cell library;
4 (b) contacting said recombinant plant cell library
5 with a detectably-labelled gene fragment produced according
6 to the method of claims 41-49 under hybridization conditions
7 providing detection of genes having 50% or greater sequence
8 identity; and
9 (c) isolating a disease-resistance gene by its
10 association with said detectable label.

1 73. A method of identifying a plant disease-
2 resistance gene comprising:
3 (a) providing a plant tissue sample;
4 (b) introducing by biolistic transformation into
5 said plant tissue sample a candidate plant disease-
6 resistance gene;
7 (c) expressing said candidate plant disease-
8 resistance gene within said plant tissue sample; and

9 (d) determining whether said plant tissue sample
10 exhibits a disease-resistance response, whereby a response
11 identifies a plant disease-resistance gene.

1 74. The method of claim 73, wherein said plant
2 tissue sample comprises leaf, root, flower, fruit, or stem
3 tissue.

1 75. The method of claim 73, wherein said candidate
2 plant disease-resistance gene is obtained from a cDNA
3 expression library.

1 76. The method of claim 73, wherein said disease-
2 resistance response is the hypersensitive response.

1 77. A plant disease-resistance gene isolated
2 according to the method comprising:

3 (a) providing a plant tissue sample;

4 (b) introducing by biolistic transformation into
5 said plant tissue sample a candidate plant disease-
6 resistance gene;

7 (c) expressing said candidate plant disease-
8 resistance gene within said plant tissue sample; and

9 (d) determining whether said plant tissue sample
10 exhibits a disease-resistance response, whereby a response
11 identifies a plant disease-resistance gene.

1 78. A purified antibody which binds specifically to
2 an rps family protein.

1 79. A DNA sequence substantially identical to the
2 DNA sequence shown in Figure 12.

1 80. A substantially pure polypeptide having a
2 sequence substantially identical to a Prf amino acid
3 sequence shown in Figure 5, (A or B).